Reducing Airborne Pathogens, Dust and *Salmonella* Transmission in Experimental Hatching Cabinets Using an Electrostatic Space Charge System

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ABSTRACT Electrostatic charging of particles in enclosed spaces has been shown to be an effective means of reducing airborne dust. Dust generated during the hatching process has been strongly implicated in *Salmonella* transmission, which complicates the cleaning and disinfecting processes for hatchers. Following two preliminary trials in which dust reduction was measured, four trials were conducted to evaluate the effectiveness of an electrostatic space charge system (ESCS) on the levels of total aerobic bacteria (TPC), enterobacteriaceae (ENT), and *Salmonella* within an experimental hatching cabinet. The ESCS was placed in a hatching cabinet that was approximately 50% full of 18-d-old broiler hatching eggs. The ESCS operated continuously to generate a strong negative electrostatic charge throughout the cabinet through hatching, and dust was collected in grounded trays containing water and a degreaser. An adjacent hatching cabinet served as an untreated control. Air samples from hatchers were collected daily, and sample chicks from each hatcher were grown out to 7 d of age for cecal analysis in three of the trials. The ESCS significantly ($P < 0.05$) reduced TPC and ENT by 85 to 93%. Dust concentration was significantly reduced ($P < 0.0001$) during the preliminary trials with an average reduction of 93.6%. The number of *Salmonella* per gram of cecal contents in birds grown to 7 d of age was significantly ($P < 0.001$) reduced by an average $\log_{10}$ 3.4 cfu/g. This ionization technology is relatively inexpensive and could be used to reduce airborne bacteria and dust within the hatching cabinet.

(*Key words*: ionizer, dust reduction, *Salmonella* reduction, pathogen reduction, hatching cabinet)

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INTRODUCTION

The introduction of large amounts of airborne fluff and dust generated during the hatching process in hatching cabinets has been shown to be one of the primary sources for *Salmonella* contamination of broilers (Bailey et al., 1992). Strong air currents in the hatcher carry the dust generated during hatch along with pathogens that may be present on or inside the eggs and recirculate them throughout the cabinet many times during the last 2 d of incubation. Eggshell fragments have also been shown to be a source of *Salmonella* contamination in hatching cabinets (Cox et al., 1990). Dust generated during the hatch in commercial hatcheries has also been implicated in pathogen cross contamination to other areas of the hatchery such as the exhaust ducts, chick room, incubators, egg room, etc. (Cason et al., 1994; Bailey et al., 1996). The importance of dust in poultry areas as a transport mechanism for potential disease-causing organisms has also been suggested by Carpenter et al. (1986). They showed that reducing airborne dust in a poultry room by a factor of two reduced airborne bacteria by a factor of 100. Leach et al. (1999) showed that *Salmonella typhimurium* could be transmitted to eggs up to 15 times more efficiently when laying hens were inoculated by an airborne route than by an oral route.

Efforts to reduce airborne transmission of disease-causing organisms in the hatcher have included air sanitation with ultraviolet light, ozone, hydrogen peroxide (Bailey et al., 1996), and formaldehyde (Sander et al., 1995a). Of the chemical treatments used in recent years, hydrogen peroxide and formaldehyde seem to be the most popular for hatcher treatment during hatching, but both have dis-

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Abbreviation Key: BGS-NAL = brilliant green agar with 200 ppm nalidixic acid; ENT = enterobacteriaceae; ESCS = electrostatic space charge system; SE = *Salmonella enteritidis*; TPC = total plate count; VRBG = violet red bile agar with 1% glucose.

advantages. Hydrogen peroxide is corrosive to metals, and formaldehyde is considered carcinogenic for humans and it damages the cilia of chicks, reducing their performance in dusty environments (Sander et al., 1995a,b).

Another option for reducing airborne transmission of dust and pathogens in hatching cabinets would be to use negative air ionization—a very old technology that reduces airborne dust and has no adverse effect on animals or humans. Negative air ionization has been shown to be effective for reducing viral transmission of Newcastle disease virus between 27 to 100% (Estola et al., 1979; Mitchell and King, 1994).

A custom electrostatic space charge system (ESCS), designed for dust and pathogen reduction in poultry areas (Mitchell and Stone, 2000), has been shown to completely eliminate airborne transmission of *Salmonella enteritidis* (SE) to the ceca of 8-d-old chicks maintained in an ion-enriched cabinet (Gast et al., 1999). ESCS also has been shown to reduce airborne levels of SE in a room with SE-infected caged layers by 95% (Holt et al., 1999), to reduce dust in hatching cabinets by 80 to 90% (Mitchell, 1998), and to reduce dust in a caged layer room 52 to 91% (Mitchell et al., 2000). The ESCS has also been shown to have a bactericidal effect within about 30 cm on airborne and surface SE (Seo et al., 2000)—reducing it by 98% or more. Preliminary trials using the ESCS within about 30 cm to treat biofilms developed on stainless-steel surfaces from poultry carcass rinses showed 99.8% reduction of bacteria within 3 h.

The objectives of this research were to determine the effectiveness of the ESCS for reducing airborne bacteria [total count, enterobacteriaceae (ENT), and *Salmonella*], *Salmonella* transmission (based on cecal colonization in chicks at 7 d of age), and dust in experimental hatching cabinets.

MATERIALS AND METHODS

Experimental Design

Each hatching trial involved 970 broiler eggs (viable at candling on Day 18) from a single flock placed into each hatcher and equally distributed from top to bottom in every other of 10 stacked plastic baskets. One hatching cabinet was used as a control, and the other identical cabinet was for ESCS treatment. Two preliminary trials were conducted to determine effectiveness of the ESCS for dust reduction as an indication of the air cleaning ability of the system. Due to limited dust concentration and particle counting equipment, the preliminary dust trials were conducted by comparing results from the treatment cabinet with the ESCS on to those in the same cabinet with the ESCS off (control) in sequential hatches. Fertilized eggs were placed in each cabinet at 18 d of age, and the chicks were removed following hatch on Day 21.

After the preliminary dust reduction trials, four trials were conducted to evaluate the effectiveness of the ESCS for reducing airborne bacteria during hatching. Numbers of bacteria (total aerobic bacteria, ENT, and inoculated *Salmonella*) were measured in the exhaust air stream of each hatching cabinet.

On Day 18, just prior to loading of the hatching cabinet, five additional fertilized eggs for each cabinet were inoculated with *Salmonella*. These seeder eggs were made by injecting 0.1 mL of a cell suspension (approximately 1,000 cells per egg) of nalidixic acid-resistant *Salmonella typhimurium* into the air cell. All five seeder eggs were placed along the basket divider in the center of the middle hatching basket. This application of seeder eggs has been shown to create a source of airborne *Salmonella* when chicks hatch out of inoculated eggs (Cason et al., 1994; Bailey et al., 1996).

In three trials, 10 chicks were taken from each cabinet. One chick was taken from each side of the top and bottom two baskets, and two chicks were taken from the uninoculated side of the middle basket. Chicks were grown to 7 d of age in Horsfall-Bauer isolation cabinets. At 7 d, the chicks were killed by cervical dislocation, and both ceca were aseptically removed and assayed for *Salmonella*. Ceca and contents were diluted at three times their weight with 1% buffered peptone. Nalidixic acid-resistant *Salmonella* were enumerated on brilliant green agar with 200 ppm nalidixic acid (BGS-NAL) by using the semiquantitative method of Bailey et al. (1988).

Egg Handling, Incubation, and Hatching Cabinets

Broiler hatching eggs were provided by Jeanna L. Wilson from breeder flocks at the University of Georgia. Nest clean eggs were collected daily, were placed onto fiberboard egg flats that were packed into cardboard egg cases, and were then held for less than 7 d at 14.4 C in the hatchery egg cooler. On the afternoon prior to setting, the seven cases that were the newest were transported to the isolation hatchery room, and flats of eggs were removed from the cases and allowed to warm to room temperature overnight. To distribute day of egg collection and egg storage length effects between the two incubators for each setting, one-half of the eggs from each egg flat were placed onto two egg incubation racks, which were placed into two separate incubation buggies, one for each incubator. The two identical, side-by-side incubators (NMC2000)[2] were operated at 37.5 C ± 0.2 C and 55% RH, and eggs were automatically turned every 2 h. The cabinets were constructed with fiberglass-reinforced plastic and were located within a negative pressure isolation room equipped with 95% efficiency exhaust filters.

Eggs were candled on Days 12 and 18 of incubation. Any egg that did not contain an easily detected viable embryo, that had a cracked shell, or that the aircell was displaced was removed and discarded at candling. After candling on Day 18, 97 eggs from each incubator were placed into each hatching basket (alternately placed on

[2]NatureForm Inc., Jacksonville, FL 32202.

the right or left side of each divided basket), for total of 970 eggs per hatcher. Baskets containing eggs were separated by an empty basket to simulate a full load of 10 stacked baskets in each hatcher. The same two incubators were transformed to hatchers and were operated at 36.9 C, and the RH was increased from 55 to 70% at 19.5 d and remained there until the hatch was removed on Day 21. The hatcher doors remained closed after *Salmonella* seeder eggs were placed into the center hatching basket on one side of the divider.

At hatch, the entire stack of hatching baskets was rolled from the ionizer hatcher while the ionizer remained on. Two chicks were removed from each basket and placed into an isolation transport box. The untreated control hatcher was then opened, and two chicks were removed from each basket and placed into a separate isolation transport box. All hatched chicks and unhatched eggs were then counted upon removal from each basket. There were no significant differences in hatchability between the control and the hatcher containing the ionizer; overall hatchability was 95.34% of eggs determined viable by candling on the Day 18 of incubation. The number of chicks that hatched corresponded to 47.7% of the total hatcher capacity. Over the four hatches, 87% of the *Salmonella* seeder eggs hatched for the control and ionizer-treated hatchers.

Electrostatic Space Charge System

A custom-designed ESCS was installed in one of the hatching cabinets, as shown in Figure 1. The system, similar to one used in other ESCS experiments (Mitchell, 1998), consisted of six ionizer bars (51 cm long) arranged in parallel and suspended near the ceiling of the hatcher and 7.6 cm below a grounded wire grid (2.5 cm × 2.5 cm welded wire) that served as an electrostatic ground plane. Each bar had downward pointing, sharp discharge electrodes spaced every 1.3 cm, and the electrodes were all supplied with –20,000 V dc. Although the system used high voltage, the current output was limited to a safe current of less than 0.5 mA. As is the case with most electrostatic charging systems, it is possible to get a harmless shock similar to touching a spark plug wire on a gasoline engine if the ionizer is touched.

The objective with this system was to transfer a strong negative electrostatic charge to airborne dust and microorganisms circulating in the hatcher during the hatching process and to collect the charged particles in grounded, metal trays filled with water and approximately 100 mL of liquid soap solution. One tray of water was suspended just above the top egg basket, and the other collection area was on the floor. The soap solution caused oil-coated fluff collected on the surface of the water to sink and maintained a well-grounded surface area available for dust collection. The ESCS was turned on immediately after 18-d-old eggs were loaded in the hatcher and was turned off after the chicks were removed on Day 21.

FIGURE 1. Picture of hatching cabinet with ionizer and collection trays immediately after loading eggs on Day 18. The ionizer unit is suspended just above the top basket, and collection trays are shown at the top and bottom. The hatching cabinet used as the control was identical, except that it did not have the ionizer or the collection trays.

Measurement of Dust Concentration and Particle Size Distribution

Dust concentration was measured at 10-min intervals, just inside the cabinet exhaust with a TSI DustTrak,[3] a laser-based instrument with a sensitivity of 0.001 mg/m^3 capable of measuring dust concentrations up to 100 mg/m^3. Particle size distributions were measured at 15-min intervals, just inside the cabinet exhaust with a Climet CI-500 Particle Counter,[4] a laser-based instrument capable of counting particles as small as 0.3 μM in six size ranges.

Air Sampling Methods for Bacteria

Duplicate air samples for bacteria were collected daily by inverting agar plates over the exhaust outlet of the hatching cabinets. Preliminary data had been previously collected to determine appropriate exposure times to allow countable plates for each type of microbiological growth media used. Brain-heart infusion agar[5] plates

[3]TSI Incorporated, Shoreview, MN 55126.
[4]Climet Instruments, Inc., Redlands, CA 92374.
[5]Becton Dickinson and Co., Sparks, MD 21152.

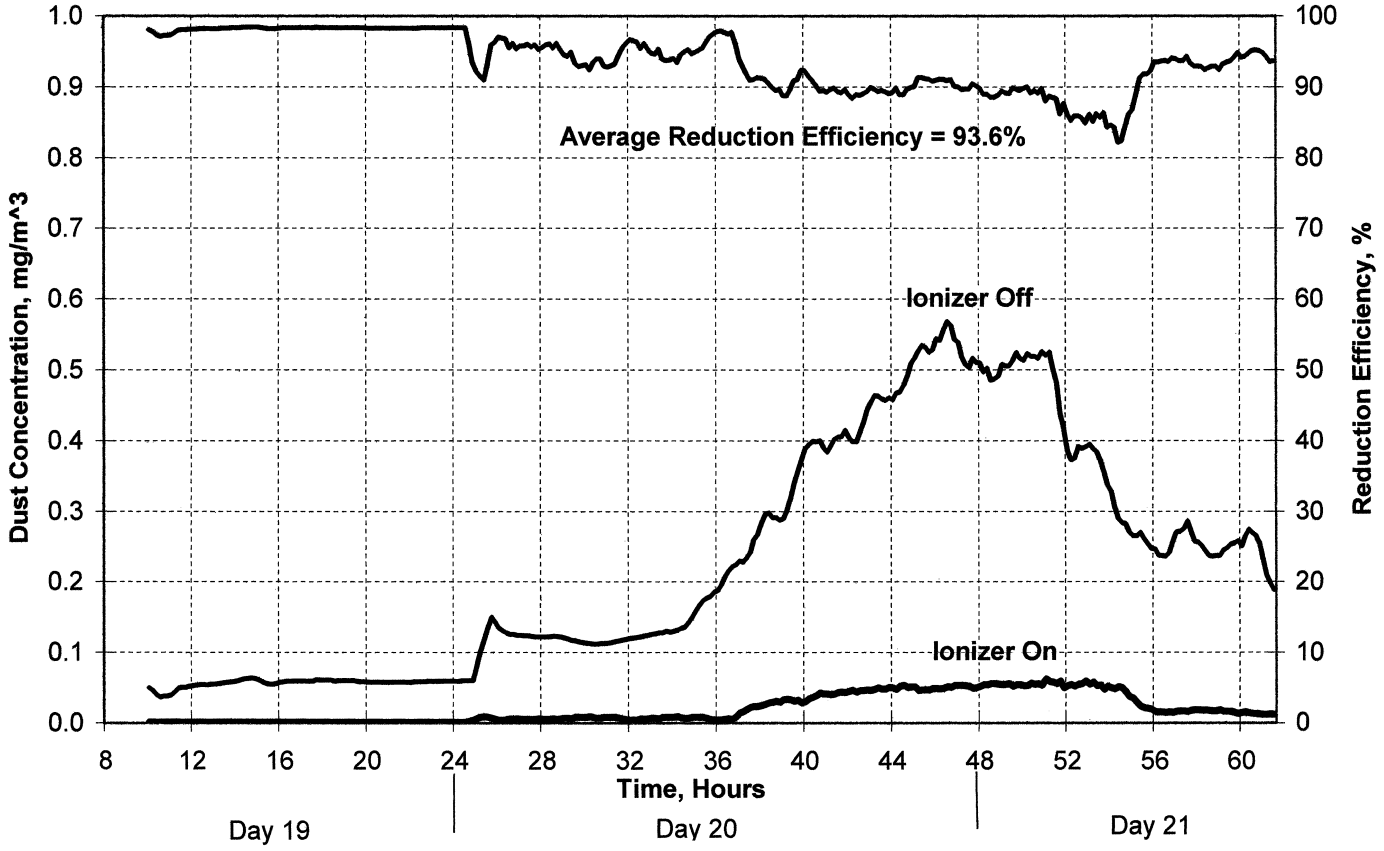

FIGURE 2. Dust concentration and reduction efficiency beginning with pip on Day 19 based on two preliminary trials—one with the ionizer and one without the ionizer. The mean dust concentration (0.020 ± SE = 0.001, n = 310) for the ionizer cabinet was significantly ($P < 0.0001$) less than that (0.229 ± SE = 0.009, n = 310) of the control cabinet.

were used for total plate counts (TPC). These plates were exposed to cabinet exhaust air for 15 s on each hatching cabinet and then incubated for 24 h at 35 C. Petri plates filled with violet red bile agar[5] with 1% glucose (VRBG) were used to assay the exhaust air for ENT. These plates were exposed to the exhaust air stream for 1 min. After the air sampling, VRBG agar plates were overlaid with VRBG agar and incubated at 37 C for 24 h. Plates with BGS-NAL were used to recover inoculated nalidixic acid-resistant *S. typhimurium.* The BGS-NAL plates were placed over the exhaust air stream for 5 min to sample for *Salmonella* and were incubated at 35 C for 24 h. Resultant colonies were counted, and characteristic colonies were confirmed as *Salmonella* with biochemical and serological tests.

Statistical Analysis

Bacterial counts were transformed to $\log_{10}$ colony-forming units and subjected to statistical analyses. Significant differences ($P < 0.05$) in treatment and control data were determined by using the Student's two-tailed *t*-test (Instat Software[6]). With the exception of the preliminary dust-reduction trials, all experiments involved a treatment cabinet and a control cabinet that were operated simultaneously. The data were analyzed for each trial. Analysis of dust data was based on two preliminary dust-reduction trials that were conducted sequentially in the same cabinet, with the ionizer on (treatment) in the first trial and the ionizer off in the second (control). The dust concentration comparisons used 310 observations taken during the last 3 d of hatch at 10-min intervals, and the dust particle count comparisons involved 208 observations taken at 15-min intervals.

RESULTS AND DISCUSSION

Dust Reduction

Figure 2 shows the effects of the ESCS treatment on dust concentration during a hatch. Dust concentration in the control cabinet increased beginning on Day 19 as pipping began and increased through peak hatching activity late on Day 20, following which it decreased until pull. Dust concentration was significantly ($P < 0.0001$) reduced in the ionizer cabinet compared to that in the control cabinet, and reduction percentages ranged from close to 100% early in Day 19 prior to pip when only ambient dust was present, to about 80% at pull on Day 21. Compared to those in the control cabinet, the ESCS

[6]Graphpad Software, Inc., San Diego, CA 92121.

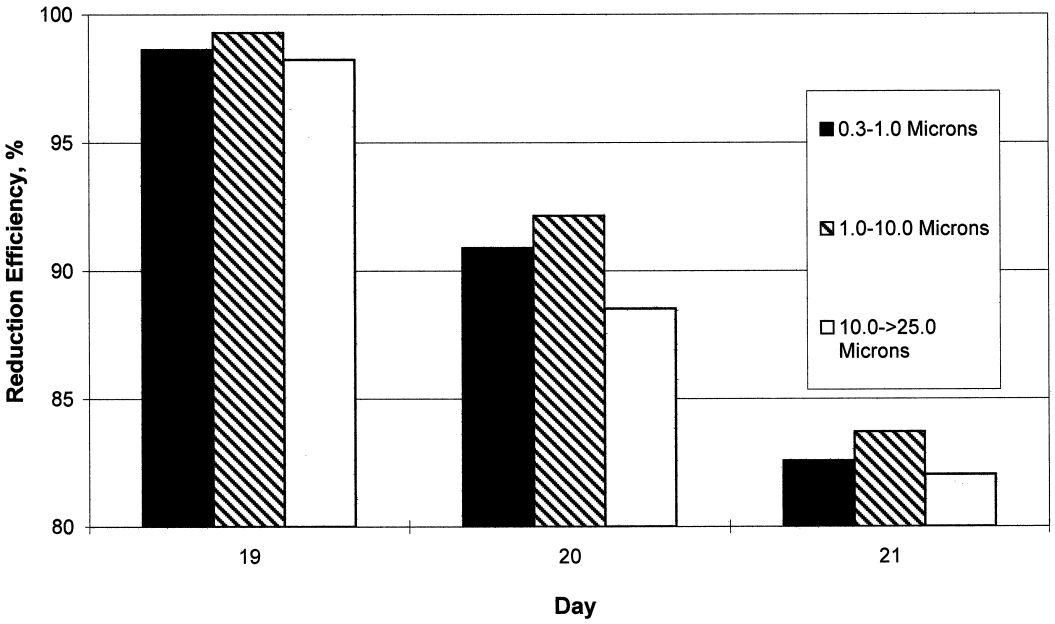

FIGURE 3. Average particle count reduction efficiency achieved with the ionizer relative to the untreated control cabinet for Days 19 to 21 for three particle size ranges based on two preliminary trials—one with the ionizer and one without the ionizer. Overall particle count reductions (based on 208 samples in each cabinet) achieved by the ionizer were significant ($P < 0.0001$) for each size range.

significantly ($P < 0.0001$) reduced particle counts for all of the particle size ranges with efficiencies ranging from an average of 98.7% on Day 19 to 82.8% on Day 21 (Figures 3 and 4). The dust concentration and particle count reductions observed for these experiments were similar to those from laboratory tests with the same ESCS using ambient particulates (Mitchell, 1998). These results are comparable to those observed in laboratory tests with 95% media filters (Mitchell, 1998) and would be expected to result in similar or greater reductions in airborne bacteria (Carpenter et al., 1986).

Reduction of Airborne Bacteria

Results of airborne bacteria measurements are shown in Figure 5. In four replications, significant ($P < 0.05$)

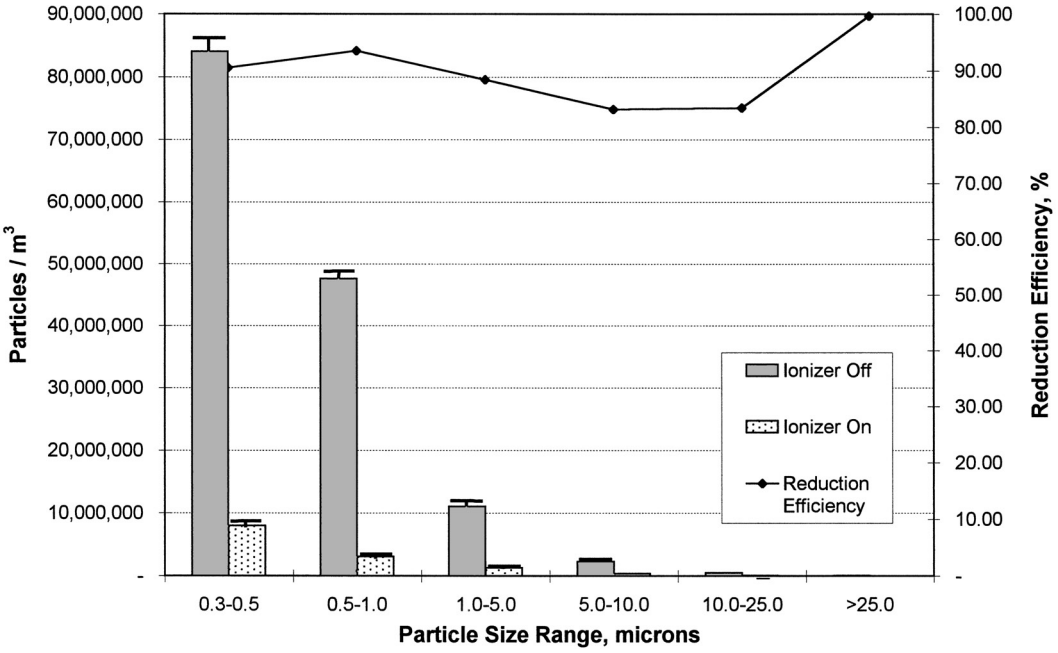

FIGURE 4. Average particle counts, standard error, and reduction efficiency with and without the ionizer for all measured size ranges for Days 19 to 21 based on two preliminary trials—one with the ionizer and one without the ionizer. Particle counts achieved by the ionizer cabinet were significantly different ($P < 0.0001$, n = 208) from those of the control cabinet for each size range.

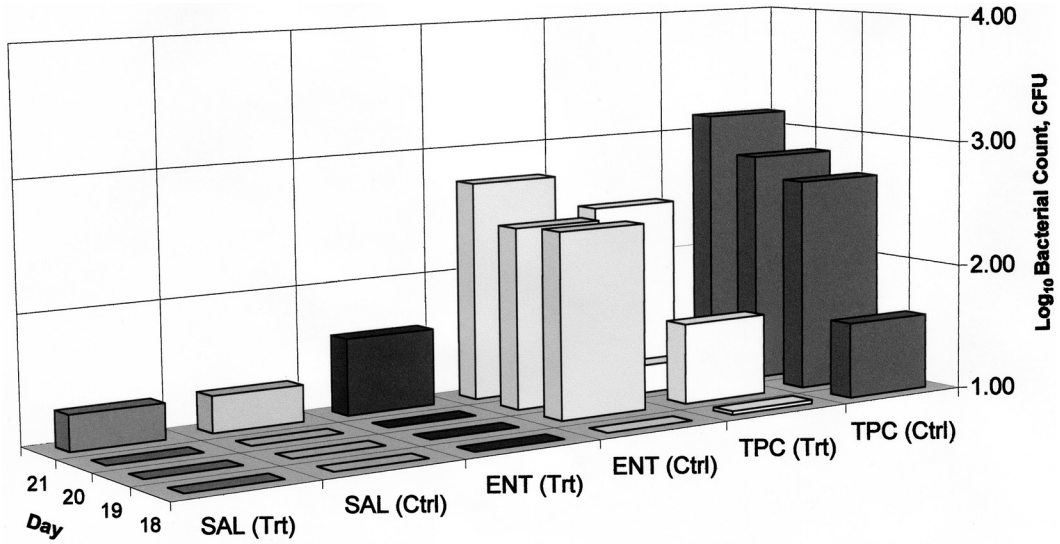

FIGURE 5. Average airborne bacterial counts (CFU) at the hatcher exhaust with (Trt) and without (Ctrl) the ionizer for four hatches from Days 18 to 21. Plate exposure time was 15 s for total plate count (TPC), 1 min for enterobacteriaceae (ENT) and 5 min for *Salmonella* (SAL). Overall differences during the hatch were significant in each hatch ($P < 0.05$) for TPC and ENT but were not significant ($P > 0.05$) for SAL.

reductions were obtained by the ESCS in TPC for which the average reduction was 0.86 logs (85.3%) and in ENT for which the average reduction was 1.33 logs (92.9%). Although airborne SAL was reduced by an average of 0.06 logs (11.9%), the reductions were not significant ($P > 0.05$). The low average counts of *Salmonella* (<1 in two of the four trials and <20 in the other two trials) were probably a factor in the statistical significance tests. The general trend of high counts for air samples of TPC, followed by moderate counts of ENT and very low counts of *Salmonella*, is common for poultry hatching cabinets. Although few *Salmonella* were recovered from air, they seem to have been sufficient to colonize the ceca of the sample chicks throughout the hatching cabinet.

There are difficulties inherent to measuring airborne bacteria by an open plate method. The dust reductions

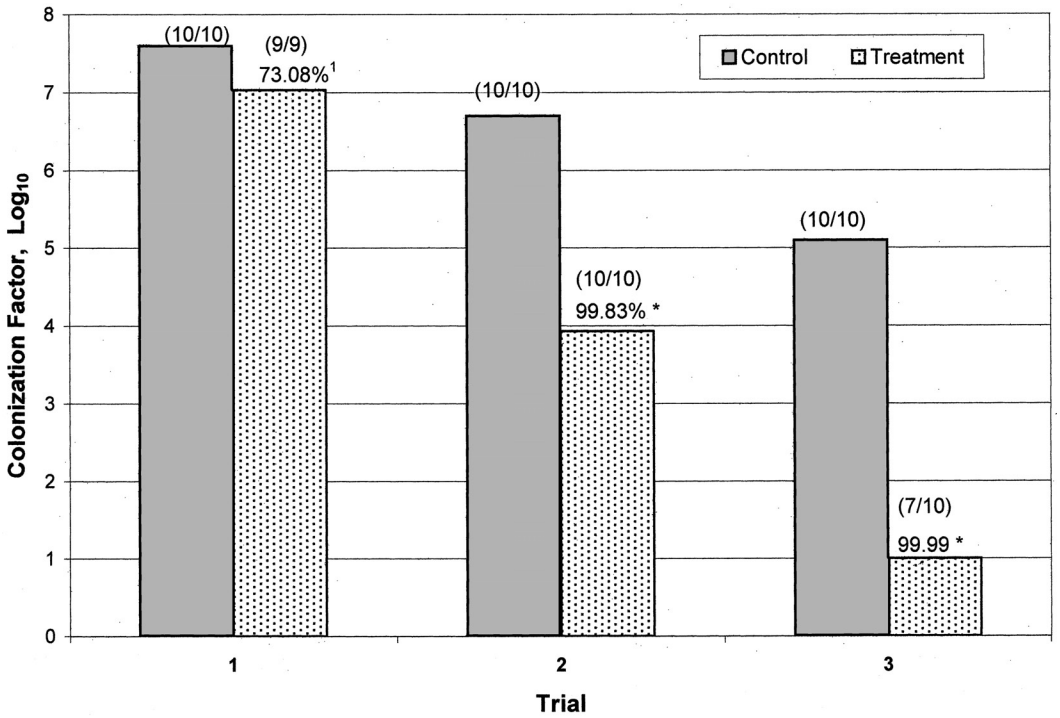

FIGURE 6. Average colonization factor (CF) results for cecas from 10 7-d-old sample chicks. Numbers in parenthesis represent the number of *Salmonella* positive chicks out of the number of chicks surviving to 7 d of age. Percentages shown on the bars represent percentage reduction in CF achieved by the ionization system. Percentages with asterisks indicate treatments that were significantly ($P < 0.001$) different from the controls.
[1]Ground plane for ionizer accidentally left ungrounded on Trial 1.

that were achieved in these experiments as well as in earlier reports (Mitchell, 1998) were very promising and comparable to those obtained with a 95% media filter. Presumably, many airborne bacteria are carried on dust. We expected to see similar reduction efficiencies for airborne *Salmonella* as those obtained for dust (Figures 2 to 4), TPC, and ENT, but the sampling methodology used has limited sensitivity for low levels of airborne bacteria. Longer sample times would increase the sampling efficiency but also would provide more time for fluff collection on the plates, which makes colony discrimination and counting more difficult.

Salmonella Transmission to Chicks

In two of the three replications in which chicks exposed to *Salmonella* during hatch were grown out to 7 d of age, the ESCS resulted in significant ($P < 0.001$) reductions in the number of *Salmonella* recovered per gram of ceca and contents (colonization factor). The colonization factors were reduced by an average of $\log_{10}$ 3.4 cfu/g by the treatment in Trials 2 and 3 in which the ionizer system was properly connected (Figure 6). The relative inefficiency of the ESCS in the first trial (Trial 1, Figure 6) was due to the ground plane (an essential part of the ESCS that strengthens the electrostatic field near the ionizer) being accidentally left ungrounded during the trial. The ability of the ESCS to significantly reduce cecal contamination in chicks in the presence of known *Salmonella*-infected chicks in a hatching cabinet suggests that airborne transmission of *Salmonella* was significantly reduced by the ESCS, as expected from ESCS studies with SE (Gast et al., 1999; Holt et al., 1999).

Several trials with a larger ESCS in full-sized commercial hatchers (approximately 15,000 egg capacity) have shown that the high efficiency dust and airborne bacteria reduction observed in the present ESCS study with relatively small experimental hatching cabinets (capacity of 1,936 eggs) can also be accomplished on a commercial scale. Results of several completed commercial trials with the ESCS will be the subject of a future report. Although the ESCS for the present trials was designed specifically for the small experimental hatcher, the basic design was used for the commercial hatcher trials with the exception that collector plates were used on the back wall of the commercial hatchers in place of the water tray collectors used in the present study. With relatively minor modification, it is expected that an ESCS could be designed for any poultry, animal, or general purpose application where it is desirable to reduce dust and pathogens in the air.

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